Supplementary Data for:

Tumour sampling method can significantly influence gene expression profiles derived from neoadjuvant window studies

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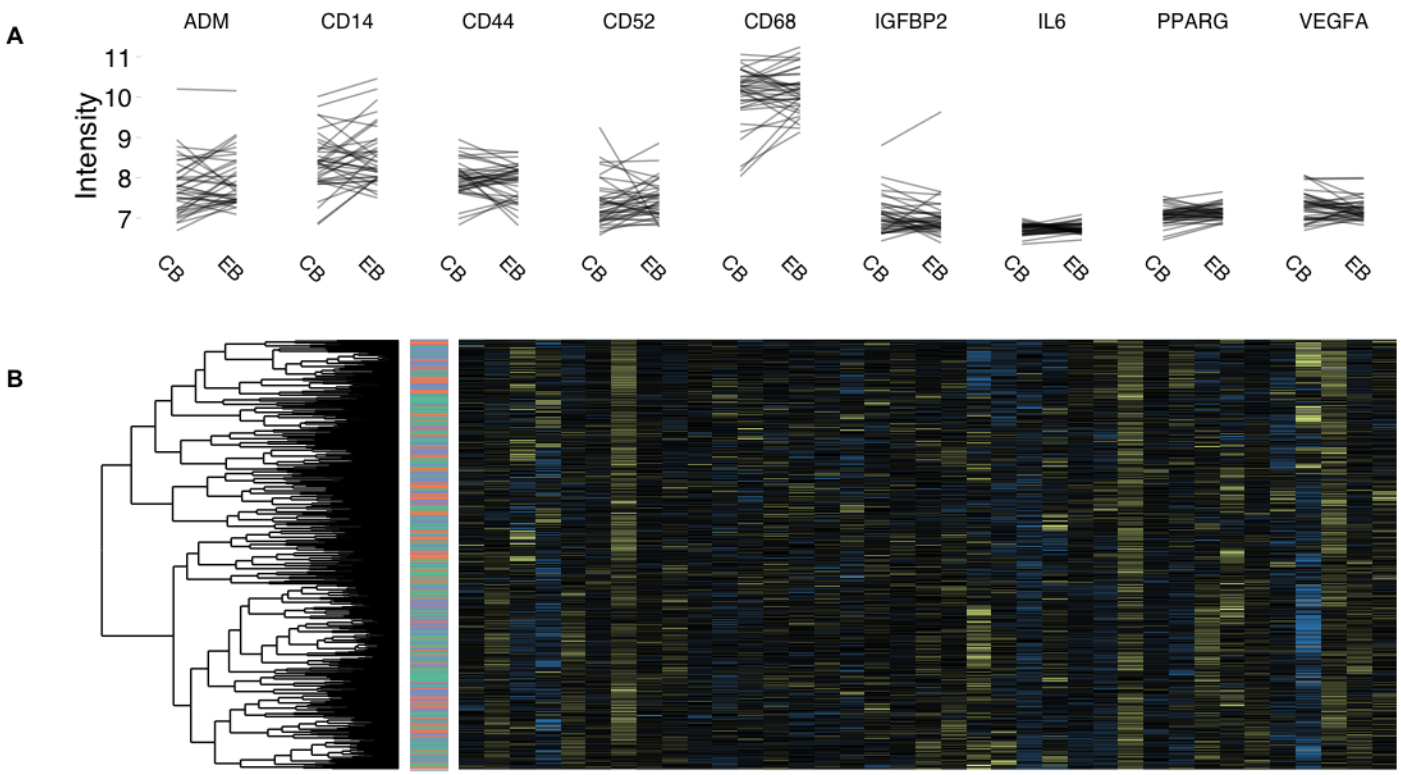
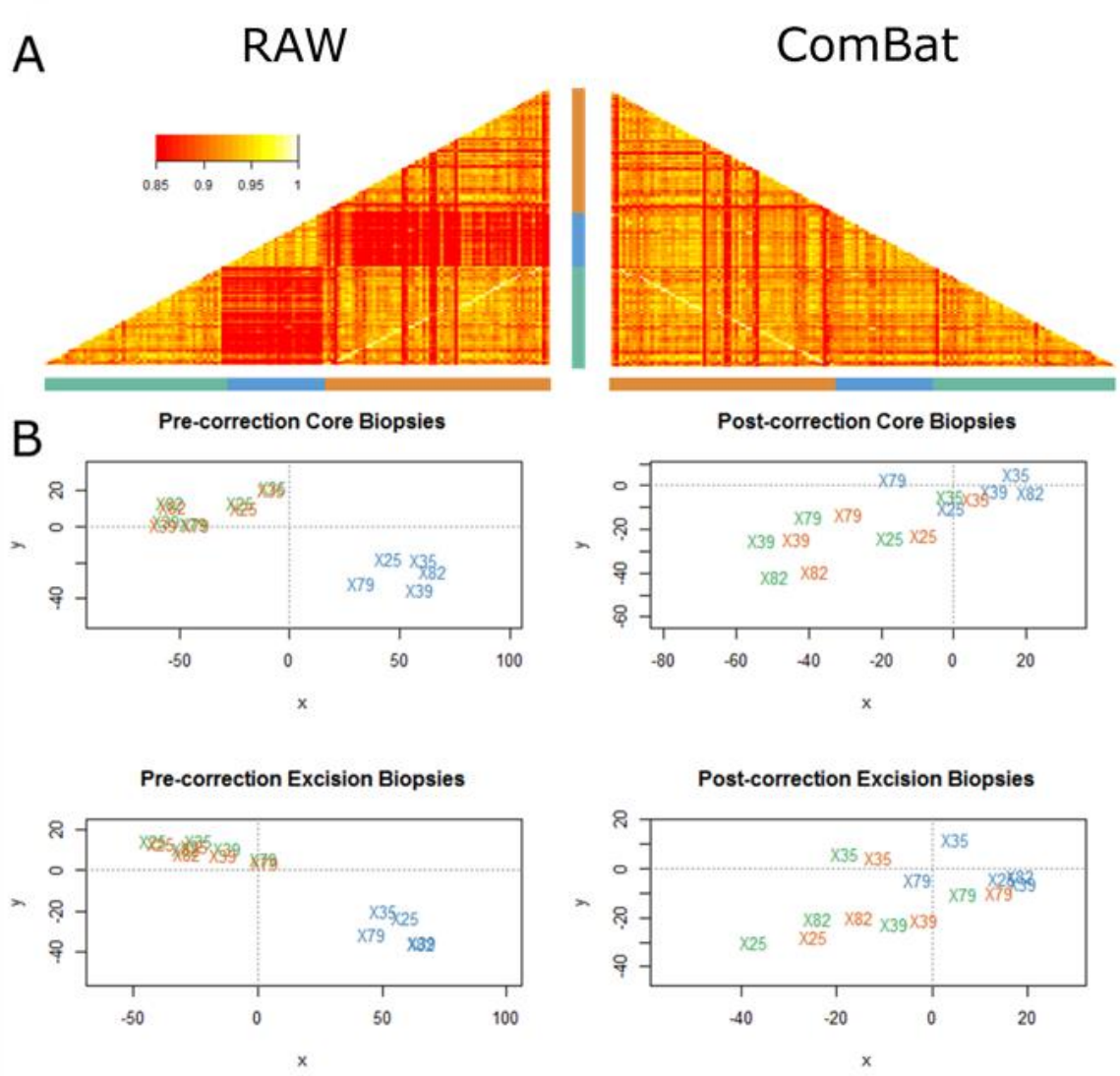
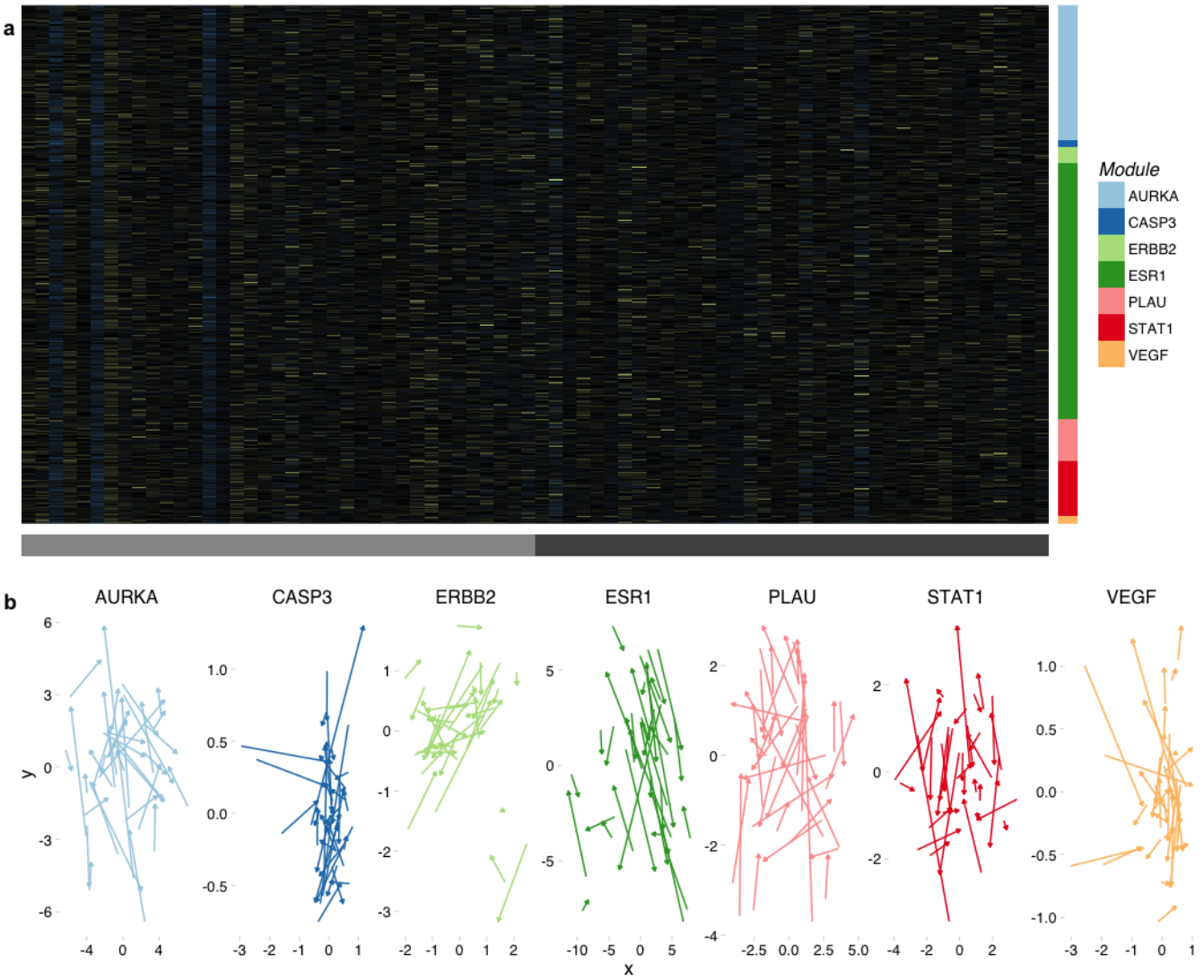


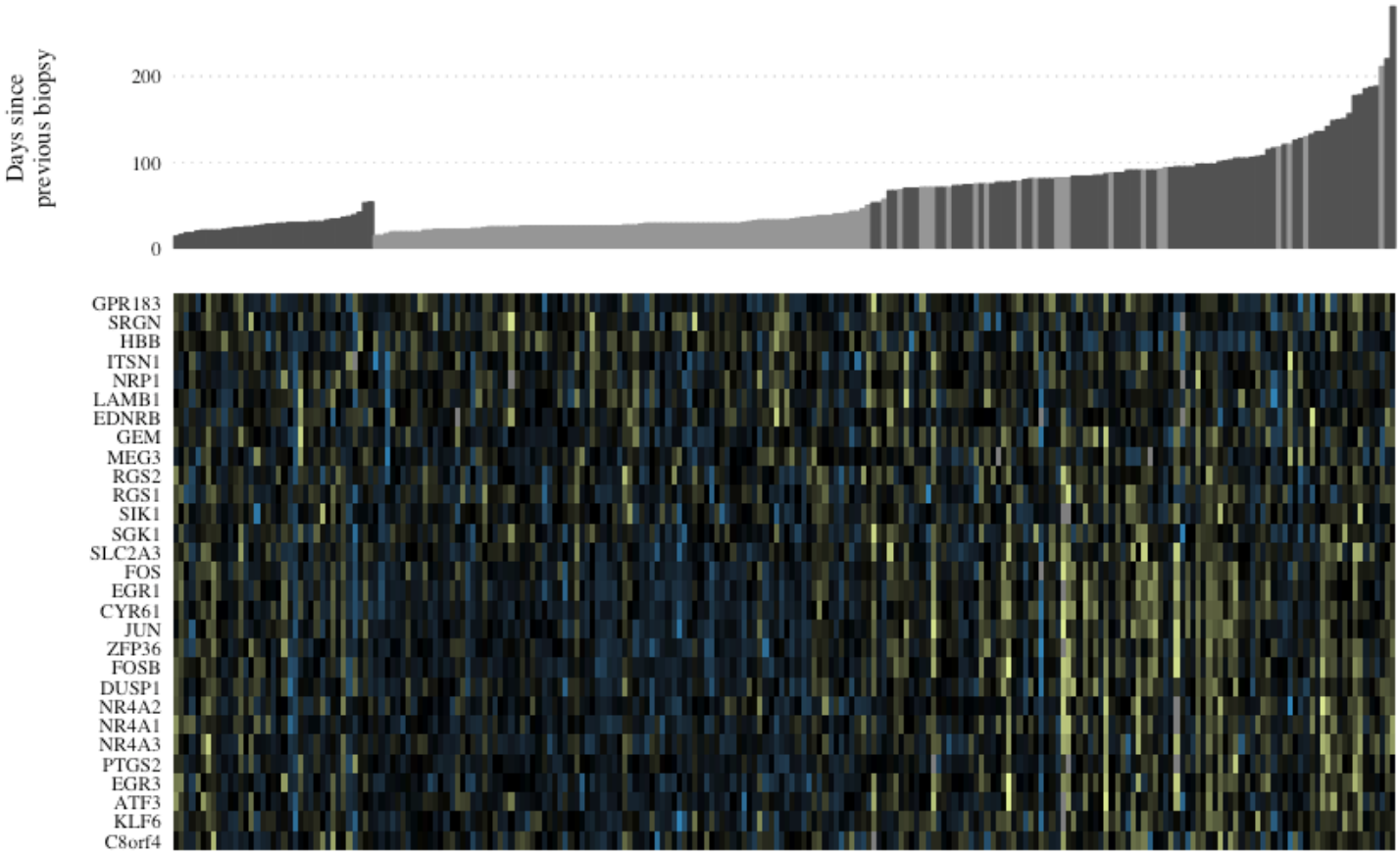
Supplementary Figure S1. Tumour sampling is independent of an immune- or wound-healing response. Top, Pairwise expression changes for 9 immune-related genes, reported by Jeselsohn et al. [1], between CB and EB NIT samples. Changes are observed to lack clear effect direction with 0/9 found to be significantly differentially regulated. Bottom, Heatmap detailing differential expression of a 589 gene wound-healing signature in NIT data, ordered by increasing biopsy time interval (Yellow = high expression, blue = low expression). Colour bar represents signature gene class – activated (blue), quiescent (green) or cell cycle (orange). Lack of any coherent gene clustering by class implies the absence of a true wound-healing response.



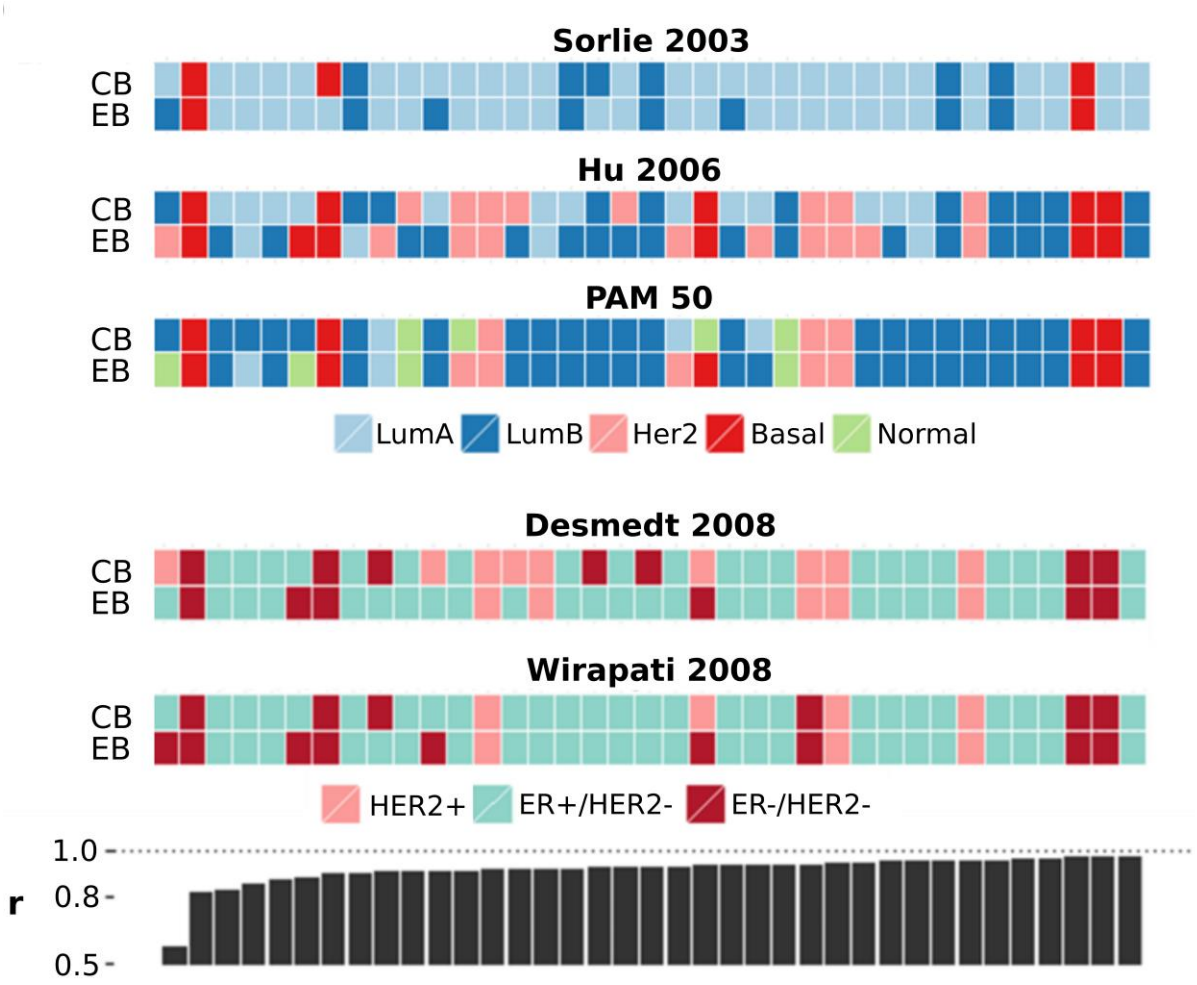
Supplementary Figure S2. Correction for dataset batch effects. RNA extraction and preparation prior to microarray hybridisation was performed using identical protocols in a single centre. However, Datasets 1 (orange) and 3 (green) were hybridised to Illumina Human HT-12 version 4 whole-genome expression bead arrays, whereas Dataset 2 (blue) hybridisation was performed on version 3 of the same platform. This resulted in an observable batch effect between uncorrected Dataset 2 and Datasets 1 & 3, when comparing sample Pearson correlations by correlation heatmap (A) or multi-dimensional scaling (B). The ComBat method was applied to remove batch effects and facilitate robust dataset integration.



Supplementary Figure S3. Effects of tumour sampling on 7 breast cancer-related expression modules. Seven gene expression modules defined by Desmedt et al.[1] were investigated to determine whether tumour sampling could affect breast cancer-related processes. (a) A heatmap compares expression (yellow = greater than row mean expression, blue = lesser than row mean expression) of core biopsied samples (light grey) with excision biopsied samples (dark grey) for the different modules (y-axis colour bar). (b) Pairwise analysis of module expression by MDS. Patient samples display a lack of uniform movement or direction as they transition from core biopsy (arrow tail) to excision biopsy (arrow head), implying a lack of a systematic breast cancer biology-related effect.



Supplementary Figure S4. Heatmap showing differential expression of NIT signature genes in NIT and letrozole treated cohorts. Colours represent gene expression fold changes (up = yellow; down = blue) between samples and their subsequent patient-matched biopsies. Samples are ordered by increasing time between biopsies. Core biopsy = grey; Excision biopsy = dark grey.



Supplementary Figure S5. Discordance in molecular subtype assignment between core and excision biopsies. Patients are ranked left to right by pairwise correlation. Colours represent SSP (Luminal A = Dark blue; Luminal B = Light Blue; Her2 = Pink; Basal = Red; Normal = Green) and SCM (HER2+ = Pink; ER+/HER2- = Turquoise; ER-/HER2- = Burgundy) subtypes.

Supplementary Table S1 - Summary clinicopathological features of the tumours in the study

IHC Status	**No. Patients**
ER+/Her2-	22 (~59%)
ER+/Her2+	8 (~21%)
ER-/Her2-	7 (~19%)
Size (mm)	
≤20	12 (~32%)
21-50	21 (~57%)
51-60	2 (~5%)
NA	2 (~5%)
Grade (Elston-Ellis)	
1	3 (~8%)
2	15 (~41%)
3	18 (~49%)
NA	1 (~3%)
Nodal Involvement	
Positive	18 (~49%)
Negative	19 (~51%)
Age (years)	
≤40	5 (~ 14%)
41-50	5 (~ 14%)
51-60	5 (~ 14%)
61-70	8 (~22%)
70-80	9 (~24%)
80+	5 (~ 14%)
Final Biopsy	
CB	0 (0%)
EB	37 (100%)

Supplementary Table S2 - Complete clinicopathological features of the tumours in the study

StudyID	Grade (Elston-Ellis)	Size (mm)	ER (Allred)	PR	Her 2	Nodes
1	3	33	0	0	2+ FISH-ve	Neg
2	1	38	7	NA	2+FISH-ve	Pos
3	3	47	6	NA	2+FISH-ve	Pos
4	2	29	8	NA	2+FISH-ve	Neg
5	3	14	7	5	3+	Neg
6	2	29	8	NA	1+	Pos
7	3	31	3	0	2+FISH-ve	Pos
8	3	15	8	NA	3+	Neg
9	2	65	8	NA	0	Pos
10	2	22	8	NA	1+	Pos
11	2	24	8	NA	3+	Neg
12	3	17	7	NA	3+	Pos
13	2	34	8	NA	1+	Neg
14	3	23	8	NA	3+	Neg
15	1	NA	0	0	NA	NA
16	2	16	8	NA	2+FISH-ve	Neg
17	2	14	8	NA	2+FISH+ve	Neg
18	3	21	7	NA	1+	Neg
19	3	20	4	0	1+	Neg
20	2	26	8	NA	3+	Pos
21	2	53	8	NA	1+	Pos
22	3	20	3	0	3+	Neg
23	NA	NA	7	NA	NA	Neg
24	3	19	8	NA	2+FISH-ve	Pos
25	2	49	0	NA	2+FISH-ve	Pos
26	3	31	0	0	2+FISH-ve	Pos
27	2	42	7	NA	0	Neg
28	3	47	0	0	1+	Pos
29	3	36	4	NA	0	Neg
30	3	25	2	0	2+FISH-ve	Pos
31	3	22	8	NA	2+FISH-ve	Pos
32	2	20	8	NA	2+FISH-ve	Neg
33	3	18	2	NA	0	Pos
34	2	12	8	NA	2+FISH-ve	Neg
35	3	37	4	0	0	Neg
36	2	21	8	NA	0	Pos
37	1	11	8	NA	0	Neg

Supplementary Table S3 - Significantly differentially expressed genes between diagnostic and core biopsies

NIT 50	Mean log2 Fold Change (EB/CB)
HBA2	-1.23
HBB	-1.17
GOLGA6A	0.08
TMEM255B	0.09
LCA5L	0.11
C20orf141	0.11
ZNF565	0.11
LRCH1	0.14
APOLD1	0.14
ABL2	0.14
FAM86FP	0.15
EDNRB	0.15
FLYWCH1	0.15
ABCA6	0.16
ITSN1	0.16
WDFY2	0.16
SPDYE3	0.18
GOLGA8K	0.19
PTGS2	0.20
KLF6	0.22
RASA3	0.22
SLC2A3P2	0.22
ATF3	0.22
GPR183	0.22
KRTAP19-6	0.23
NR4A3	0.24
NPIPA3	0.24
SIK1	0.25
NR4A2	0.26
ZSWIM4	0.30
NRP1	0.31
LAMB1	0.36
SRGN	0.36
C8orf4	0.39
SGK1	0.45
EGR3	0.52
GEM	0.53
SLC2A3	0.59
RASD1	0.61
MEG3	0.64
JUN	0.75

RGS1	0.76
NR4A1	0.77
ZFP36	0.83
CYR61	0.97
RGS2	1.02
FOS	1.13
EGR1	1.48
DUSP1	1.56
FOSB	1.60

Supplementary Table S4 - Cross-table comparing IHC subtypes and PAM50 subtype assignments in the diagnostic core biopsy samples

	Basal	**Her2**	**LumA**	**LumB**	**Normal**
ER-/Her2-	0.21	0.07	0.43	0.00	0.29
ER+/Her2-	0.05	0.11	0.66	0.09	0.09
ER+/Her2+	0.25	0.13	0.56	0.06	0.00

References

1. Desmedt, C. *et al.* Biological processes associated with breast cancer clinical outcome depend on the molecular subtypes. *Clin. Cancer Res.* **14,** 5158–65 (2008).